## Remarks

Claims 1-19 were present in the application as filed. By preliminary amendment filed with the initial application papers, claim 6 was canceled and claim 20 was added, thereby resulting in pending claims 1-5 and 7-20. Claims 1-5 and 7-20 remain pending in the application.

The present invention provides a method of removing bacterial endotoxin from a specific type of pharmaceutical process solution, that is, one which contains an amphiphilic pharmaceutical drug or vaccine. Amphiphilic substances, particularly viral surface antigens, such as influenza surface antigens, present special challenges with respect to endotoxin removal since it is believed that, due to both having amphiphilic structures, the antigens and endotoxin become strongly associated under aqueous conditions.

To address this specific situation, the method of the present invention comprises treating the solution with an ionic surfactant at a concentration effective to dissociate the endotoxin from the amphiphilic pharmaceutical drug or vaccine substance in the solution. The resulting solution is then filtered through a molecular weight cut-off filter that has a pore size effective to retain the amphiphilic pharmaceutical drug or vaccine substance but allow the dissociated bacterial endotoxin and ionic surfactant to pass through.

Claim 1 is amended herein to clarify that the method of the present invention comrises a first step of treating the solution with an effective concentration of ionic surfactant to dissociate the endotoxin from the amphiphilic pharmaceutical drug or vaccine in the solution followed directly thereafter by filtration of the solution through a molecular weight cut-off having a pore size effective to retain the amphiphilic pharmaceutical drug or vaccine but allow the dissociated bacterial endotoxin to pass through, without the need for an intervening step, for example a protein precipitation step.

Claim Rejection Under 35 USC §112, second paragraph

Claims 1-5 and 7-20 are rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Office Action alleges that claims 1-4 are rendered vague and indefinite by the use of the terms "vaccine substance" and "vaccine antigen." Accordingly, claims 1-4 are amended herein to clarify that the pharmaceutical process solution contains a "vaccine" containing an "antigen," for example, a viral antigen.

Withdrawal of the rejection under 35 U.S.C. §112, second paragraph is respectfully requested.

Claim Rejection Under 35 USC §103(a)

Claims 1-5 and 7-20 were rejected as being unpatentable over Shanbrom (EP 0 083 999) in view of Shanbrom (U.S. Patent 4,315,919).

As described above, the present invention addresses the specific problem of separating amphiphilic pharmaceutical substances from bacterial endotoxins which are also amphiphilic. A known problem with amphiphilic substances is that they can form strong associations with endotoxins and it is believed that complexes may be formed between endotoxins and amphiphilic substances. Consequently, it is very difficult to separate the two without employing methods that adversely affect the amphiphilic drug or vaccine. This is particularly true in the case of certain vaccines, for example, influenza vaccine, where the amphiphilic vaccine antigens are assembled into complexes (*e.g.*, rosettes). In the specific case of influenza antigen, it is believed that endotoxin is incorporated into the haemagglutinin/neuraminidase rosettes.

Applicants' solution to this problem, as provided by the claimed invention, is to treat the process solution with an **ionic** surfactant, and preferably, an anionic surfactant, so as to dissociate the endotoxin from the amphiphilic drug or vaccine substance. The resulting solution is then subjected to ultrafiltration such that the larger amphiphilic drug or vaccine complex is retained by the filter, while the smaller, dissociated endotoxin fragments and surfactant pass through the filter.

As previously discussed, the depyrogenation method of Shanbrom ('919) while it suggests the use of any amphiphilic surfactant including ionic and non-ionic surfactants, provides only examples using a **non-ionic** surfactant, Triton-X 100 (See Examples 1-5)and includes a protein precipitation step. Furthermore, Shanbrom ('919) contains a disclaimer with respect to the use of ionic surfactants, such as sodium deoxycholate.

Specifically, Shanbrom ('919) teaches (at column 4, lines 10-29) that the use of amphiphiles such as sodium deoxycholate "have not been previously suggested as able to produce irreversible disaggregation of endotoxins such as to make them practical for the treatment of plasma protein products which are to be used for human administration..." because their use results in reversible disassociation of endotoxin as long as the amphiphile is present. This is in agreement with Applicants' statement on page 17 of the specification that it was previously believed that sodium deoxycholate treatment of antigen solutions followed by filtration through a molecular weight cut-off filter membrane as a means of separating endotoxin from vaccine preparations could not be done on a large scale.

Shanbrom's disclosure further suggests (but does not demonstrate), that ionic amphiphiles are effective in removing endotoxin when used in conjunction with protein precipitation methods to destroy the endotoxins prior to their separation and removal along with the amphiphile in the supernatant (sol. 4, lines 20-29).

Applicants' believe that given this statement regarding ionic surfactants, one of skill in the art would not be motivated to combine its teachings with the Shanbrom (EP) which only teaches depyrogenation using non-ionic surfactant.

The method of Shanbrom (EP) teaches treatment of a variety of substances including depryogenation of an experimental **non-proteinaceous** polysaccharide (therefore, **hydrophilic**) vaccine, heparin and the antibiotic, dehydrostrptomycin sulfate using an aqueous solution of Triton 100-X , a **non-ionic** surfactant, followed by **precipitation** with polyethylene glycol 6000 or extraction with $CHCl_3$. Depyrogenation of human serum albumin with Triton 100-X followed by ultrafiltration is also disclosed.

Though Shanbrom (EP) teaches depyrogenation of an aqueous albumin solution using a **non-ionic** surfactant followed by filtration, in view of his statement in Shanbrom ('919) with respect to ionic surfactants and the reversibility of the disassociation of endotoxin from the material to be treated, Applicants' respectfully submit that one of skill in the art would not be motivated to combine the Shanbrom references. Thus it would not be obvious to person of ordinary skill, based on the Shanbrom references, either alone or in combination, to use an **ionic** surfactant to remove pyrogen from an **amphiphilic** pharmaceutical drug or vaccine and then filter the solution to remove the ionic surfactant from the pharmaceutical drug or vaccine without the additional precipitation step suggested by Shanbrom ('919).

As has been shown herein, therefore, the deficiencies of Shanbrom (EP) are not cured by the addition of the teachings of Shanbrom ('919), and even with the combination, the present invention is not achieved. Shanbrom (EP) does not disclose use of an ionic surfactant and Shanbrom ('919) seems to teach away from the use of ionic surfactants without a protein precipitation step.

For all the foregoing reasons, Applicants submit that the claimed invention is clearly distinguished from the prior art and respectfully request that the rejection under §103 be withdrawn.

There being no further issues, the application (including claims 1-5 and 7-20) is believed in condition for allowance and such action is courteously requested. Although no fee is believed due at this time, the Commissioner is authorized to charge any deficiency in fee that may be considered to be due in connection with the filing of this paper to Deposit Account 08-1935.

Respectfully submitted,

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